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**THE DECENTNIAL PUBLICATIONS OF
THE UNIVERSITY OF CHICAGO**

THE DECENTNIAL PUBLICATIONS

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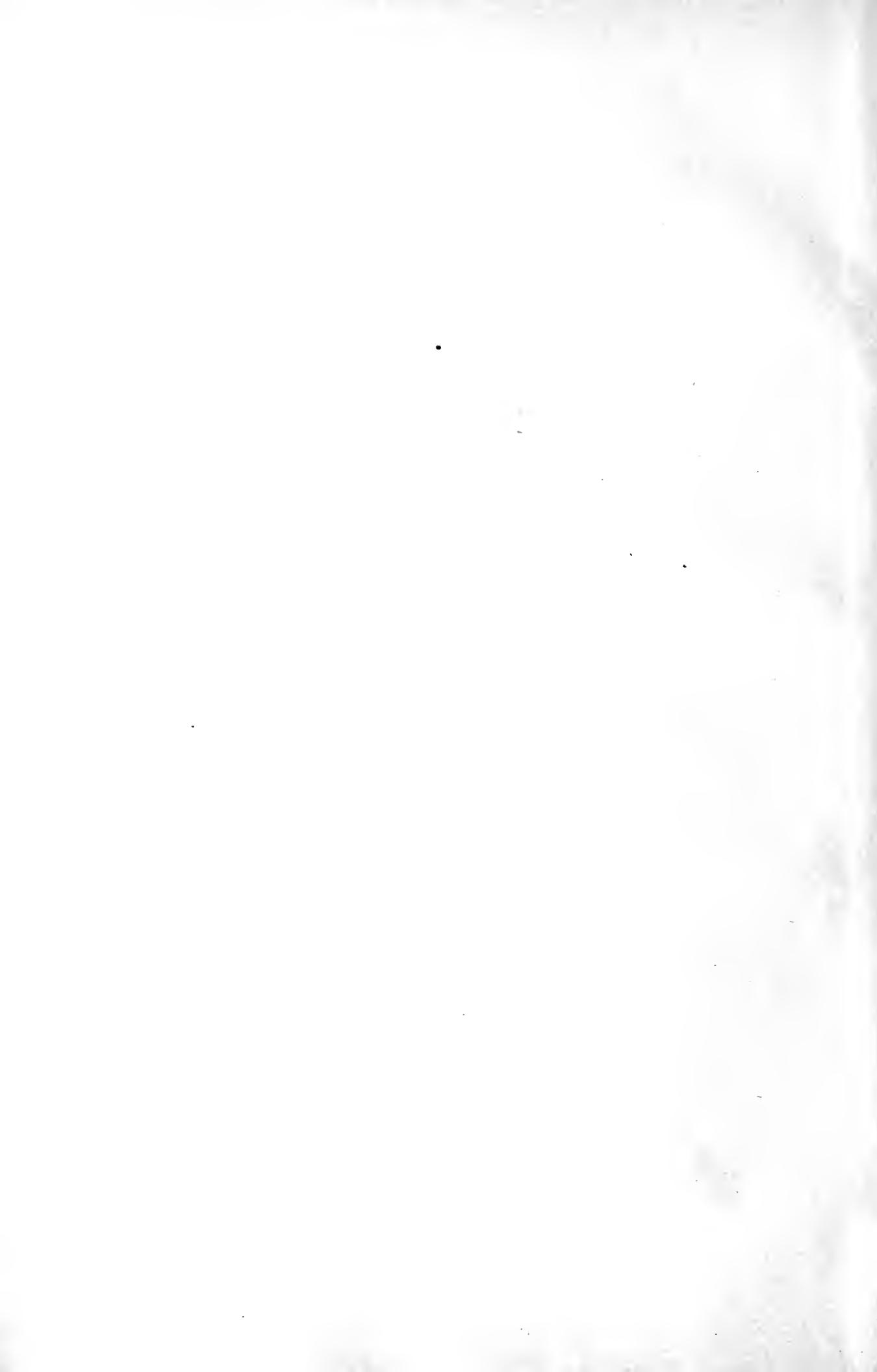
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THESE VOLUMES ARE DEDICATED

TO THE MEN AND WOMEN
OF OUR TIME AND COUNTRY WHO BY WISE AND GENEROUS GIVING
HAVE ENCOURAGED THE SEARCH AFTER TRUTH
IN ALL DEPARTMENTS OF KNOWLEDGE

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THE DECENTNIAL PUBLICATIONS
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MITOSIS IN PELLIA

In 1901 Davis (7) made a detailed study of mitosis in various phases of the life-history of *Pellia*. Centrospheres were found during the early divisions in the germinating spore, but could not be identified in the sporophyte or in later stages of the development of the gametophyte.

At this time it hardly seems desirable to make a more extended résumé of the literature, since it is still too incomplete and indefinite to warrant generalizations. In presenting our own results, we shall occasionally refer to the preceding papers and also to papers dealing with mitosis in other groups.

MATERIAL AND METHODS

Most of the material for this work was collected near Bonn in Melbthal and in the Siebengebirge. Early in October the spore mother-cells of *Pellia* are already quite deeply lobed, and occasionally a sporogonium is found in which the spores are already formed. By the middle of November nearly all of the spore mother-cells have divided and many of the spores have germinated. The winter of 1901-2, in the Rhine Province, was a very mild one, and germination proceeded with only occasional interruption throughout the entire season. Material brought into the laboratory at any time after the middle of November developed much more rapidly than in the open, and would shed the spores within a week or ten days.

Before placing the material in the fixing agents, the calyptra was dissected away and about one-third of the sporogonium cut off with a razor, thus freely exposing the spores. In a few cases the mass of spores, held together only by the elaters, was removed from the sporogonium, but while not nearly so many spores were lost as might be anticipated, this tedious method was found to be unnecessary, since the other process readily yielded smooth sections as thin as 2μ or 3μ .

Several fixing agents were used, but only two gave thoroughly satisfactory results. These were chromo-acetic acid (0.8g. chromic acid, 0.5c.c. glacial acetic acid, 100c.c. water) and a modification of Flemming's solution (0.5g. chromic acid, 0.5g. glacial acetic acid, 1 per cent. osmic acid 10c.c., water 100c.c.). While achromatic structures stain more readily after solutions containing some osmic acid, equally good preparations were often obtained from material fixed in the former solution.

Most of the sections were cut at 2μ or 3μ , but sections 5μ , and even 10μ or 15μ , in thickness were used in determining the number of asters and in counting chromosomes.

Haidenhain's iron alum haematoxylin, with or without a slight tinge of erythrosin, Congo red, or orange G, gave fairly satisfactory preparations, but gentian violet proved to be so much superior in differentiating kinoplasmic structures that safranin and gentian violet, sometimes with the addition of orange G, were used in most of the work. Sections were stained, usually over night, in safranin (1g. safranin in 100 c.c. of 50 per cent. alcohol), then washed in 50 per cent. alcohol until all red color was removed from the achromatic structures, and then stained for one or two hours in gentian violet.

(saturated aqueous solution). From the gentian violet the preparations were transferred directly to absolute alcohol, where they were quickly dehydrated, the process being hastened by moving the slide to and fro in the alcohol. Clove oil was used for clearing. The clove oil should be rinsed off with good cedar oil, otherwise the gentian violet gradually fades. When orange G was used the preparations were taken from the gentian violet, dipped a few times in water, stained for one minute in orange, and then transferred to the absolute alcohol.

In studying the preparations hollow glass globes, filled with various solutions, served as ray filters and condensers. A light blue solution of ammonia copper sulphate was used for most of the work, but occasionally a light violet solution of permanganate of potash, imitating the gentian violet stain, gave a sharper differentiation of the kinoplasmic structures.

While the work deals chiefly with the first three divisions of the germinating spore of *Pellia epiphylla*, and in these divisions is largely confined to the centrospheres, asters, and spindle, mitosis was studied in other phases of the life-history of this genus, and also in several other liverworts, among which were *Conocephalus*, *Marchantia*, *Aneura*, *Pallavicinia*, *Scapania*, *Lophocolea*, and *Porella*.

The principal results of the investigation were presented in a *Vortrag* before Professor Strasburger and the advanced students of the Bonn laboratory in February, 1902, and in July of the same year a brief résumé was presented before the botanical section of the American Association for the Advancement of Science.

THE SPORE MOTHER-CELL

The spore mother-cell was observed in *Pellia epiphylla*, *P. calycina*, *Aneura multifida*, and in *Porella platyphylla*. In all of these forms the nucleus occupies a central position during the development of the lobes which are to become spores. It seems probable that the nucleus is concerned in the formation of the lobes. We found nothing to support Davis's (7) statement that the nucleus lies in one of the lobes until shortly before the first division of the mother-cell. No quadripolar spindles, like that described by Farmer (7) for *Pallavicinia*, were found in any of the above-mentioned forms. On the contrary, the four spores in all these cases are formed by two successive divisions, as described by Farmer (10) and by Davis (7) for *Pellia epiphylla*. Unfortunately, no material of *Pallavicinia* in this stage was available, but the striking resemblance of Farmer's (8) figures to the mitoses in deeply lobed mother-cells of other Jungermanniales leads us to suggest, as Davis (7) has already done, that Farmer (8) may have misinterpreted the quadripolar figure in this genus.

THE GERMINATING SPORE

The first, second, and third mitoses in the germinating spore of *Pellia* cannot be regarded as distinct types, for with diligent searching one could select a series of mitoses at the second division, or even at the third, which would be identical with a

series at the first division. In fact, we have used Figs. 7, 8, and 19 of the third mitosis to illustrate also the same stages in the first and second mitoses. Nevertheless, it is true that, in a great majority of cases, kinoplasmic activity is most energetic during the first division, and that in succeeding divisions it becomes less and less conspicuous until centrospheres and asters cease to attract any attention, and it finally becomes doubtful whether they are present.

THE FIRST MITOSIS IN THE GERMINATING SPORE

As the nucleus of the germinating spore increases in size preparatory to the first division, the area immediately surrounding it becomes comparatively free from starch grains and coarser granules (Plate XXV, Fig. 1). It seems reasonable to suggest that some substance, escaping from the nucleus into the cytoplasm, causes this zone and acts as a stimulus to the formation of the extra-nuclear portions of the achromatic figure. It is not impossible that such a substance might actually take the form of a centrosphere. (The origin of the aster will be considered when dealing with the second division.) After the spirem has become segmented into chromosomes the nucleus elongates and the nucleolus appears very much vacuolated (Fig. 2). At this stage a pair of dome-shaped caps (Figs. 3, 4) may be recognized at opposite poles of the nucleus. These caps, which will be considered later, appear in transverse section as a delicate ring, but a similar section of the completed spindle shows a dense mass of fibers (Fig. 5).

During the earlier prophases the poles of the spindle are usually rounded (Figs. 3, 4, 6), but, as the metaphase approaches, the caps (Figs. 3, 4) which have given the poles of the spindle a rounded form become resolved into fibers, and the poles may vary in shape from sharply pointed figures, like that shown in Fig. 10, to such broad, indefinite ones as those shown in Fig. 8 (Plate XXV) and Fig. 27 (Plate XXVII). Spindles with three and even more poles are not very rare. They do not originate like the multipolar spindles of the spore mother-cells of vascular plants, but are preceded by the bipolar condition or are formed through the influence of three or more centrospheres or asters (Plate XXVI, Fig. 16, Plate XXVII, Fig. 23). During the anaphases the poles of the spindle are sometimes sharp and sometimes indefinite.

In the prophases it is plain that the achromatic figure is made up of the asters and two half-spindles (Fig. 6). As the spindle continues to develop, some of the fibers—the mantle fibers—become attached to the chromosomes; the other fibers increase in length until they reach the opposite pole, thus forming a part of the central spindle.

While the poles are separating from each other, radiations are easily seen, and they continue to be fairly conspicuous until the spindle has reached its full length, when they rapidly disappear, losing their staining capacity first at the peripheral ends, then throughout their entire length, and finally becoming indistinguishable. When the metaphase is reached, the radiations have usually disappeared (Fig. 7), and during the anaphases, while the chromosomes are passing to the poles, it is very seldom that any trace of radiations can be found. In the telophases, however, the radiations

reappear, but are not centered in any single point. When the nuclear membrane begins to form, the radiations again become indistinct and disappear as before. Just before the spindle reaches its full length (Fig. 6), the radiations often attain their greatest prominence, sometimes appearing as extremely coarse strands. In nearly all cases, even in very thin sections, some of the rays can be traced from the pole of the spindle to the *Hautschicht*. The diameter of the rays is usually greater at the polar end, but a slight increase in diameter at the *Hautschicht* also is not uncommon. The rays are usually simple, but may be branched especially during the earlier stages.

It is worthy of note that the radiations are most pronounced and stain most deeply with gentian violet, while the nucleus is elongating and its poles are separating from each other; and, further, that during this period many of the radiations connect the poles with the *Hautschicht*. The explanation which we venture to suggest is that the radiations take an active part in separating the poles from each other. The fact that the radiations disappear as soon as the poles have reached their widest separation supports this hypothesis. The reappearance of the rays in the telophase does not seem to be so definitely concerned with movement, because they again disappear before the nucleus has perceptibly changed its position: still, it is possible that there may be a slight movement of the nucleus toward the center of the new cell. The reappearance, however, takes place as the nuclear membrane begins to be formed, and it may be an expression of kinoplasmic activity during the formation of a *Hautschicht* surrounding the nuclear membrane, or the rays may be contributing to the formation of the nuclear membrane itself, which, we believe, is largely kinoplasmic in its nature.

THE SECOND MITOSIS IN THE GERMINATING SPORE, WITH REMARKS ON APICAL CELL, ANTHERIDIA, NUCLEOLI, AND CHROMOSOMES

The second mitosis is remarkably easy to fix and stain; so that, while the first mitosis, if equally well prepared, might show the early prophascs with a little more clearness, our material afforded a better study of these stages during the second mitosis.

In studying the second mitosis, special attention was devoted to the centrosphere and to the origin of the achromatic structures. The terms "centrosome" and "centrosphere" are frequently confused. Until much more is known about the origin of these structures and their relation to each other, it is hardly worth while to attempt any definitions. A typical centrosphere—as the term is used in this paper—is shown in Fig. 12 (Plate XXVI). The centrosphere consists of the same substance as the astral rays and the spindle fibers. The elongated body toward which the rays converge in Fig. 15 is also a centrosphere, and the densely staining masses at the poles of the spindle in Fig. 6, although not organized into a definite body, consist of the same material as centrospheres and, at an earlier stage in mitosis, may have had a more definite form. We have not intended to represent a *centrosome* in any of our figures. Bodies which have the superficial aspect of centrosomes are shown in Figs. 14, 16, and 17, but here the sharply staining body at the center of the centrosphere is, without doubt, the cut end

of an astral ray. The structure at the upper pole in Fig. 9 certainly looks like a centrosphere containing a centrosome, but such an appearance is so rare that it seems safer to regard the sharply staining body as a chance granule. Still, it is evidently just such a body as this that Van Hook (38), in his recent study of *Marchantia*, interprets as a centrosome.

In the very early prophanes a beautiful system of radiations becomes quite conspicuous. This system we regard as an aster, comparable with the asters of Thallophytes and of animals. The system first appears as a few fibers converging to a point which is usually in contact with the nuclear membrane or very near to it (Figs. 11–13), but, in some instances, may be at a considerable distance from the nucleus (Figs. 14–16). Persistent search failed to reveal any body which could be identified positively as a centrosome or centrosphere before the appearance of the aster, and even after the appearance of the aster and centrosphere, no centrosome could be distinguished. Granules, like those shown in all the figures, were frequently found in contact with the nuclear membrane after the nucleus had begun to enlarge, and it is probable that some of the granules were centrospheres, although no method was found for identifying them before the appearance of the rays. Bodies which bear remarkable resemblance to centrosomes (Figs. 14, 16, 17) and which, for a time, were interpreted as genuine centrosomes, proved to be merely the cut ends of coarse fibers. Sometimes several deeply staining points may be seen; such an appearance might easily be mistaken for a centrosphere containing several granules. In cases like those shown in Figs. 14–17, the "granules" are, without doubt, nothing but the cut ends of fibers. The two centrospheres in Fig. 17 are practically alike, but the one at the upper pole is represented in median section and the other in surface view, the fibers in vertical view appearing as dots. However, it must be admitted, that occasionally the deeply staining points are really granules (Fig. 9), but the cases are so rare that we have not regarded such granules as a functional part of the mitotic mechanism.

After a study of the germinating spore had failed to show any centrosomes, the nuclear figures were examined in other phases of the life-history, particularly in the apical cell and its younger segments, and in the developing antheridia. The apical cell and the rapidly dividing cells near it are quite favorable for study. The character of the mitoses in this region is represented in Figs. 9 and 10. The lower pole in Fig. 9 shows the more usual condition, although the rays are frequently as strongly developed as those shown at the upper pole, a considerable number of the rays reaching to the *Hautschicht*. A careful examination of this figure will show that there is no definite centrosphere like those in Figs. 12 and 13. In later stages (Fig. 10) the spindle becomes sharply bipolar and the radiations disappear.

The antheridia were examined with particular interest because Schottländer (30) had reported centrosomes during all stages in the development of the antheridium of *Marchantia*, and Belajeff (2) had found blepharoplasts throughout the development of the spermatogenous cells of *Marsilea*. However, nothing which could be interpreted

as a centrosome was found in our material, which furnished a series from the initial cell up to stages in which more than thirty cells appear in a transverse section of the antheridium. Unfortunately, the material showing the last two or three divisions preceding the formation of the spermatozoid mother-cells was not satisfactory, and, consequently, no positive statement can be made in regard to blepharoplasts, although we should assume them to be present during the last one or two mitoses.

In the germinating spore a differentiated area, already described as a centrosphere by Farmer (9), Strasburger (33), and by Davis (7), is often found at the center of the aster. The origin and behavior of this structure, which we regard as a genuine centrosphere, are rather puzzling. While we assumed that it must appear earlier than the rays, and that the rays were developed from it, the failure to identify the structure before the appearance of the rays, and its frequent absence when it might be expected to be present, led to a careful study of the subject. The conclusion was reached that the centrosphere gives rise to the rays, but that the rays may also contribute materially to the substance of the centrosphere.

Although we have not been able to make any satisfactory study of living material, we believe that appearances warrant the theory that there is a streaming movement in the rays. Such a theory is not entirely new to zoologists. If the theory be true, when the streaming is toward the nucleus the centrosphere would increase in size, while a continued streaming toward the periphery would cause the centrosphere to disappear. In regard to the origin of the rays, nothing more definite was determined. Finely granular areas, showing a tendency to stain with gentian violet, were sometimes seen in earlier stages, but the actual formation of rays or centrospheres from these areas could only be surmised. These areas do not seem to differ essentially from those which we (4) have already observed accompanying the male nuclei of *Pinus Laricio*. In some of Miss Ferguson's (13) figures of the same species and of *Pinus rigida* the areas approach the form of definite centrospheres. The aster appears so suddenly that its mode of development is largely conjectural. In a fully developed aster, there is usually an increase in the diameter of the ray at the centrosphere (Plate XXVI, Figs. 13 and 16), and occasionally a slight enlargement at the *Hautschicht*. An enlargement of the ends of the rays, as shown in Fig. 13, is just what should be expected if there is a streaming of material. The variability in the size of the rays and their irregularly granular character also favor the theory that they are lines of streaming material. The tendency of small nucleoli or microsomes to collect on the rays, as pointed out by Schaffner (27) in his study of *Lilium*, and as is familiar to all who have seen mitoses in the embryo-sac of *Lilium* and similar forms, is another argument in favor of this theory.

The asters arise at opposite poles of the nucleus, but not simultaneously. Serial sections of a large number of nuclei were examined before this conclusion was reached. We can hardly understand Davis's (7) statement that in his studies he "has never found a nucleus with a clearly defined solitary aster beside it. This is a very important point and the search was persistent." In our own preparations of the second and

third mitosis we never found anything but the solitary aster in the earliest stages. In studying this point, reconstructions were made from thin sections, and series were cut thick enough to include the entire nucleus. It is true that the first aster does not usually reach its fullest development before one appears at the opposite pole. In Figs. 14 and 15 and also in Fig. 19 (third division) there is only one aster. However, the second aster usually appears before development has proceeded so far. In spite of the fact that the two asters do not arise simultaneously, we can confidently support Davis's (7) conclusion that the two asters do not arise by the division of a single one. We found only two preparations in which the asters were less than 180° apart, except in case of tripolar figures, which were not very rare (Fig. 16, Plate XXVI—third pole not shown—and Plate XXVII, Fig. 23). In early stages the two poles usually differ from each other in appearance, one pole being rather pointed and the other comparatively blunt (Plate XXVII, Figs. 21, 22, 24, 25). Cases like Fig. 21 indicate that the blunt pole has been the last to develop. At this stage, neither pole is sharp, both being more or less rounded. The dome-shaped prominences or "caps," as they may be called, are by no means easy to interpret. In some cases the cap looks like a mere extrusion of the nuclear membrane, while in others the nuclear membrane is still intact after the caps have become quite conspicuous. The rounded ends indicate considerable pressure from beneath. That the cap is something more than a structure built up by fibers radiating from the aster is shown by its appearance and by the fact that in transverse section it presents a continuous line. The cap becomes finely granular and suggests a delicate membrane being resolved into fibers, rather than a membrane being formed from fibers (Fig. 4). In our opinion, the cap is derived from the outer portion of the nuclear membrane, or is itself a delicate layer—a sort of *Hautschicht*—immediately surrounding the nuclear membrane. The caps do not seem to be different from those seen in the root tips, as described by Némec (24), Schaffner (28), and others.

The rays of the aster do not penetrate the caps, but are closely applied to them. The aster exerts a strong pull, as may be seen during the period of elongation, although the elongation is due, in some degree, to pressure from within.

As in the first mitosis, the spindle in early stages consists of two half-spindles (Fig. 26). Until the caps become resolved into fibers they keep the spindle rounded (Fig. 26). The caps generally break up into fibers during the metaphase or early anaphases, and the poles of the spindle may then become blunt or irregular (Fig. 27). Occasionally the caps keep the poles of the spindle rounded even after rather late anaphases have been reached (Fig. 28).

The polar radiations generally disappear at the end of the prophases, are absent during the metaphase and anaphases, and reappear in the telophase (Figs. 26–29). That portion of the spindle which lies between the two caps is undoubtedly nuclear in origin. It consists of a very dense mass of spindle fibers which appear with remarkable suddenness (Fig. 20; cf. Fig. 5).

From observations on the nucleolus, we feel sure that this body contributes consid-

erable substance to the growing chromosomes. As the chromosomes increase in size, the nucleoli become more and more vacuolated, and material which resembles that of the nucleoli is often found adhering to the growing chromosomes. After the chromosomes have reached their full size, the nucleoli fragment, the fragments usually staining with gentian violet. Soon the entire nuclear cavity becomes filled with granular matter staining with gentian violet, and at this period the central portions of the spindle appear suddenly as the granular matter disappears. A few early spindles were noted in which this central portion did not seem to consist of sharply defined fibers. While such an appearance is often due to faulty methods, the sharply defined fibers in other figures in the same preparation favor the inference that these undifferentiated portions represent stages in the transformation of nucleolar matter into spindle fibers. In our opinion, these phenomena support Strasburger's (33) theory that the nucleolus contributes some of the material for the spindle.

Observations on the chromatin were merely incidental, but it is certainly safe to say that *Pellia*, in spite of the small size of its nuclei, is a favorable object for such study. As has just been mentioned, the nucleolus probably contributes something to the substance of the chromosomes. Although the chromosomes are small, they can be distinguished very early and seem to lose their identity much later than is usually the case. Mitoses in the venter of the archegonium show a longitudinal splitting of the chromosomes before the breaking down of the nuclear membrane, while in the germinating spores the splitting occurs much later.

The number of chromosomes in the gametophyte, as counted in the germinating spores and in the actively dividing region of the thallus, is eight. This number, however, is far from being constant. Both Farmer (10) and Davis (7) report occasional irregularities. In the present study, a few nuclei were found with only seven chromosomes, and nine chromosomes were counted in more than a dozen cases (Plate XXVI, Fig. 20). Long spindles upon which the chromosomes are irregularly arranged are not infrequent, and it seems probable that such a mitosis might result in an unequal distribution of the chromosomes, and thus account for variations from the typical number (Plate XXV, Fig. 8).

THE THIRD MITOSIS IN THE GERMINATING SPORE

While considerable attention was given to the third mitosis, an extended description is hardly necessary. Prominent asters (Plate XXVI, Fig. 19) like those of the two preceding mitoses are often present, but they are frequently absent, and the caps appear with only a few radiations (Fig. 18) or even none at all. There are no radiations in the metaphase (Plate XXV, Fig. 7). In short, it is possible to select from the third mitosis a series of stages identical with a typical series from the apical region of the thallus. At the fourth and succeeding mitoses the resemblance to the usual vegetative divisions becomes more and more pronounced, while asters and centrospheres become correspondingly rare.

THE CENTROSOME PROBLEM

The centrosome² problem is one of extreme difficulty, and perhaps the difficulty is greater for the botanist than for the zoologist. At least, the difficulties are different in the two cases. That there are in animals well-defined centrosomes which function as organs of nuclear division, all investigators agree, and animals or tissues in which centrosomes do not occur are regarded as exceptions. The existence of the organ is not a serious problem; rather, the more recent investigations have sought to establish the permanent or transitory character of an organ which all admit to be present during mitosis. In plants, on the other hand, even the existence of a centrosome is a problem which must be considered separately for the different groups.

It is of interest to note that centrosomes in plants were first observed in diatoms in 1886 by H. L. Smith (31). When Guignard in 1891 published his classic paper on fertilization, botanists at once accepted the results and confirmatory accounts appeared. Strasburger (33) found centrosomes in *Larix*, Humphrey (18) in *Psilotum*, Mottier (22) in *Delphinium*, Schaffner (26) in *Alisma* and *Sagittaria*, Campbell (3) in *Equisetum*, Lauterborn (21) and Karsten (20) in diatoms, and other investigators reported centrosomes in various forms ranging from the algae up to the flowering plants. In fact, the centrosome seemed to be as universally present in plants as in animals. Belajeff (1) and Farmer (11), however, failed to find centrosomes in *Lilium*. At the same time Strasburger (35), directing a remarkable group of investigators, attacked the problem in all the principal groups of plants. Those who studied Thallophytes found centrosomes, but those who studied Pteridophytes and Spermatophytes not only found no centrosomes, but, in tracing the origin of the multipolar spindle, they found conditions which seemed to preclude any such bodies. Just as the discovery of centrosomes was followed by confirmatory accounts, the multipolar spindle and the non-existence of centrosomes in the vascular plants received immediate confirmation. Guignard, Schaffner, and others still continued to find centrosomes in flowering plants, although these bodies, as represented in the figures, became noticeably less conspicuous than in earlier accounts. In Guignard's (15) recent studies of fertilization no centrosomes are represented in the figures, and no reference to any such structures is made in the text, even during the stage at which the famous "quadrille of the centers" was formerly (14) described. The fact that the great majority of cytologists, with the most approved technique and provided with apochromatic immersion lenses fail to find centrosomes in flowering plants, added to the fact that the mode of spindle-formation both in reproductive and in vegetative cells does not require the participation of a centrosome, makes the evidence overwhelming that the centrosome, as an organ of division, does not exist in this group.

In regard to the Pteridophytes, the evidence is similar, but not nearly so extensive. The blepharoplasts of Pteridophytes and Gymnosperms will be considered later.

² In referring to flowering plants no attempt has been made to distinguish between centrosomes and centrosphere. In describing mitosis in liverworts some writers have used these terms indiscriminately.

In the mosses the centrosome problem has received no serious attention, doubtless on account of the small size of their nuclei. Whether there is even a blepharoplast or not, still remains to be determined.

In the liverworts, no centrosome is found at any stage in the life-history. However, in *Pellia* and *Conocephalus*, and perhaps in all forms with such extensive intrasporal development of the gametophyte, a centrosphere appears during the early divisions in the germinating spore, but even in these few divisions the centrosphere is very transitory, not persisting from one nuclear division to the next, and appearing only irregularly during the division with which it is concerned. Still, this transitory centrosphere is a functional part of the mitotic figure during the first two or three divisions. In *Pellia*, at the fourth division, the centrosphere may or may not appear, and in subsequent divisions it was only rarely that we could identify the body at all.

Among the Thallophytes, sharply defined centrosomes have been described by competent observers who are thoroughly familiar with all phases of the centrosome problem.

In the fungi, judging from Harper's (16) work on various Ascomycetes, a centrosome is present during the period of free nuclear division in the ascus, when it functions in the formation of the spindle. After the period of free nuclear division, the centrosome behaves in a very peculiar manner in forming the young wall of the ascospore.

The centrosome has received more attention in the algae than in the fungi. In papers by Farmer and Williams (12), and by Strasburger (34), centrosomes are described in the oogonia and segmenting eggs of *Fucus*.

During the early segmentations of the fertilized egg, Strasburger (34) was able to observe the division of the centrosome and to trace its continuity from one cell to another. In the development of the oogonium, however, no such continuity could be recognized. In the large apical cell of *Stypocaulon*, Swingle (37) found that the centrosome divides, giving rise to the two centrosomes from which the spindle is developed. He was able to recognize the centrosome even during the resting-stage of the nucleus. In the tetraspore mother-cell of *Dictyota*, Mottier (23) found comparatively large and somewhat elongated centrosomes. These bodies divide and, at least during divisions in the tetraspore mother-cell and in the early divisions of the germinating tetraspore, persist from one cell-generation to another. They develop asters and play an important part in the formation of the spindle.

Lauterborn (21) figures conspicuous centrosomes in *Surirella* and other diatoms. Karsten (20) also describes centrosomes in diatoms, and his beautiful preparations, which it was our pleasure to examine, show these bodies as sharply defined as in most animal mitoses. Both Lauterborn and Karsten agree that a centrosome, or at least a body derived from it, becomes cylindrical or ring-shaped, and functions as a spindle during mitosis. The centrosomes of diatoms stain intensely and are not surrounded by a centrosphere. Lauterborn found centrosomes even during the resting condition

of the nucleus and cell, but Karsten was not able to identify the body positively until the radiations began to appear. Davis (5) describes a centrosphere, but no centrosome, in the tetraspore mother-cell of *Corallina*. The centrospheres give rise to the spindle, and consequently play an essential part during nuclear and cell division. No centrospheres could be recognized during the resting-stage of the nucleus.

Thus it appears that in many of the algae well-defined centrosomes are present, at least during certain phases of the life-history, and that the centrosomes may divide and persist from one cell-generation to another, while in other algae the centrosome does not show such a degree of permanence. In the algae which we have mentioned the centrosomes are not surrounded by a clear area. In *Corallina* it is to be noted that there is no centrosome, but only a centrosphere. In none of the algae have centrosomes been traced throughout the life-history of the plant. In some fungi centrosomes are present during the mitoses concerned in the development of spores. Among the liverworts we doubt whether there is, at any period in the life-history, a centrosome like those described for the Thallophytes. The centrosphere appearing and functioning during only a few mitoses, has replaced the functional centrosome.

The polar radiations which are often conspicuous during mitosis in Pteridophytes, Gymnosperms, and Angiosperms, are of the same nature as those of Thallophytes and bryophytes, but in the higher groups (and, possibly, in most mitoses in the lower groups) a definite centrosome, or even a centrosphere is lacking. Centrosomes and centrospheres in vascular plants have been described and figured so frequently by such competent observers that he would be rash, indeed, who would claim that all such accounts have no foundation except in perverted imagination and preconceived theories. That theories suggested by the accounts of zoologists and supported principally by misinterpretations of plant structures have caused exaggeration in the drawings and descriptions of botanists is probably true. While we believe that most of these centrosomes are to be interpreted as chance granules, nucleoli, pieces of chromosomes, etc., still we see no reason why a centrosome or centrosphere might not occur occasionally through atavism. The finely granular areas which have been noted during spermatogenesis in Coniferales and the similar areas which are often seen in Angiosperms, are, in our opinion, vestiges representing historically the centrosphere as it appears during the early mitoses in the germinating spore of *Pellia*.

The blepharoplasts described for various Pteridophytes and Gymnosperms are, in our opinion, to be interpreted as centrosomes. It seems to be true that in *Ginkgo* (17), *Cycas* (19), and *Zamia* (39) they appear only in the body cell and in the spermatozoids. In *Marsilea*, however, Shaw (29) traced them another cell-generation farther back, and in the same genus Belajeff (2) found blepharoplasts even during the earlier stages in the development of spermatogenous tissue. But, granting that the blepharoplast appears during only one or two cell-generations, this does not seem to be a valid argument against its centrosome character, for in *Pellia* the centrosphere is clearly distinguishable during only a few mitoses, and even in the multicellular Thallo-

phytes, if the centrosome should prove to be present throughout the life-history, it is at least much more conspicuous at some phases than at others. Plants furnish numerous illustrations of the gradual reduction, and even the disappearance of organs during phylogeny. Most botanists admit that in the earliest sporophytes all the cells were sporogenous; but, during phylogeny, portions of the sporogenous tissue became sterilized until the sporogenous tissue finally became much limited in extent and now appears only during a few cell-generations. During such reductions, functions of cells or organs may become completely changed, as in the case of the elaters of liverworts, which are, historically, sporogenous cells and often develop like sporogenous cells, even up to the spore mother-cell stage. In the formation of the ascospore, the function of the centrosome is not the same as during the mitotic divisions in the ascus. Other examples might be cited.

That the function of the blepharoplast is somewhat peculiar must be admitted. Radiations, however, and spindle fibers, which are often the most conspicuous accompaniments of centrosomes and centrospheres, are actively concerned in movement and are not essentially different from the radiations or cilia of blepharoplasts. In form and function centrosomes present so much diversity among themselves that the peculiarities of the blepharoplast need occasion no surprise. One has only to compare the typical spherical centrosome with the rod-like centrosome of *Dictyota*, the hollow cylindrical spindle of some diatoms, and with the centrosome which forms the *Hautschicht* of the ascospore.

We should conclude, therefore, that centrosomes, centrospheres, and blepharoplasts are historically related, and with their radiations, spindle fibers, and cilia are only different manifestations of kinoplasmic activity, movement in all cases being the principal function.

Pellia, with the prominent aster and centrosphere of its germinating spore becoming less and less distinct in succeeding mitoses until a condition is reached resembling that which prevails in the flowering plants, presents in its own life-history a great reduction of the aster and the disappearance of the centrosphere.

I am deeply indebted to Professor Strasburger for his kindly courtesy and helpful suggestions during my work in his laboratory.

SUMMARY

1. The principal part of the work deals with the first three divisions in the germinating spore of *Pellia epiphylla*. We have not intended to attack the excellent work of previous investigators, but rather have attempted to extend a little farther a knowledge of the phenomena of mitosis.

2. A centrosphere, but no centrosome, is very prominent during early prophases of the first mitosis in the germinating spore. The centrosphere is not present at all during the subsequent stages of mitosis. An aster is also conspicuous during the early prophases of the first mitosis, but disappears before the metaphase is reached.

Radiations reappear during telophases. The aster is believed to be concerned in separating the poles of the spindle; the radiations during the telophase may be concerned in forming the nuclear membrane or a *Hautschicht* about the nuclear membrane. In the second and third mitoses, the centrospheres and asters become more and more indistinct, and in succeeding mitoses the centrosphere becomes indistinguishable, and a few irregular rays replace the aster.

3. The rays are believed to be lines of streaming material, consisting of the same substance as the centrospheres.

4. Centrosomes, centrospheres, and blepharoplasts are believed to be the same structures historically, being only different manifestations of a common kinoplasmic activity.

5. No centrosomes or centrospheres were found during mitoses in the apical region of the thallus or in developing antheridia. It was not determined whether a blepharoplast occurs toward the close of spermatogenesis.

6. The central portion of the spindle is believed to be derived in large measure from the nucleolus.

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EXPLANATION OF PLATES

All figures were made with a Bausch and Lomb camera lucida, Zeiss apochromatic immersion objective 2 mm. 1.30 N. A., and Zeiss compensating ocular 12; magnification, about 1,500 diameters. All figures are from *Pellia epiphylla*, Raddi, except Figs. 1, 2, 11, and 12, which are from *Pellia calycina*, Nees.

PLATE XXV

(Figs. 1-8, mitoses in germinating spore; 1-6, first mitosis, 7 and 8 third mitosis; Figs. 8 and 10, mitosis in apical region of thallus.)

FIG. 1.—Area about the elongating nucleus has become rather free from starch grains and larger granules. Asters and caps are present.

FIG. 2.—Peculiar aster at upper pole; the papilla indicates that it is pulling upon the nuclear membrane.

FIG. 3.—The cap is very conspicuous and the nuclear membrane is still intact.

FIG. 4.—A cap just beginning to break up into fibers. A transverse section at this stage shows a ring.

FIG. 5.—Transverse section of fully formed spindle.

FIG. 6.—Mitosis in late prophase; the spindle is evidently made up of two half-spindles, radiations conspicuous; definitely formed centrospheres are lacking.

FIG. 7.—Metaphase of third mitosis; no radiations or centrospheres are present. Figures of first mitosis are the same at this stage.

FIG. 8.—Irregular mitosis (third mitosis) in an unusually large spore, suggesting how nuclei with an irregular number of chromosomes might be formed.

FIG. 9.—Mitosis near apical cell; caps prominent and radiations reaching to the *Hautschicht*; at the upper pole is a granule resembling a centrosome.

FIG. 10.—Anaphase in mitosis near the apical cell; no asters or centrospheres are present.

PLATE XXVI

(Figs. 11-17, second mitosis; 18-20, third mitosis, in germinating spore.)

FIG. 11.—Very early prophase.

FIG. 12.—Centrosphere and radiations.

FIG. 13.—Very prominent centrosphere and radiations. The centripetal ends of the radiations have a pseudopodium-like aspect and suggest that the radiations are lines of streaming material.

FIG. 14.—Centrosphere in which the cut end of a fiber resembles a centrosome. There is no centrosphere or aster at the other pole.

FIG. 15.—Irregular, elongated centrosphere with prominent aster; no centrosphere or aster at the other pole.

FIG. 16.—Tripolar spindle, the third pole not shown. The cut end of a fiber resembles a centrosome. The pull upon the nucleus is evident; upper aster at some distance from the nucleus.

FIG. 17.—The two centrospheres are practically alike, but the upper one is shown in median section, while the lower one appears in surface view, the fibers having the appearance of granules within a centrosphere.

FIG. 18.—The more usual appearance of an early prophase at the third mitosis; prominent caps, but no centrospheres or very definite aster.

FIG. 19.—An exceptionally prominent centrosphere and aster at the third mitosis; no centrosphere or aster at the other pole.

FIG. 20.—Transverse section of mitotic figure at the third mitosis, just before the splitting of the chromosomes, showing nine chromosomes.

PLATE XXVII

(Figs. 21-9, second mitosis in the germinating spore.)

FIG. 21.—Cap more prominent at upper pole; nuclear membrane intact.

FIG. 22.—Nuclear membrane has broken down at the poles, but is still intact at the sides of the nucleus.

FIG. 23.—Tripolar figure.

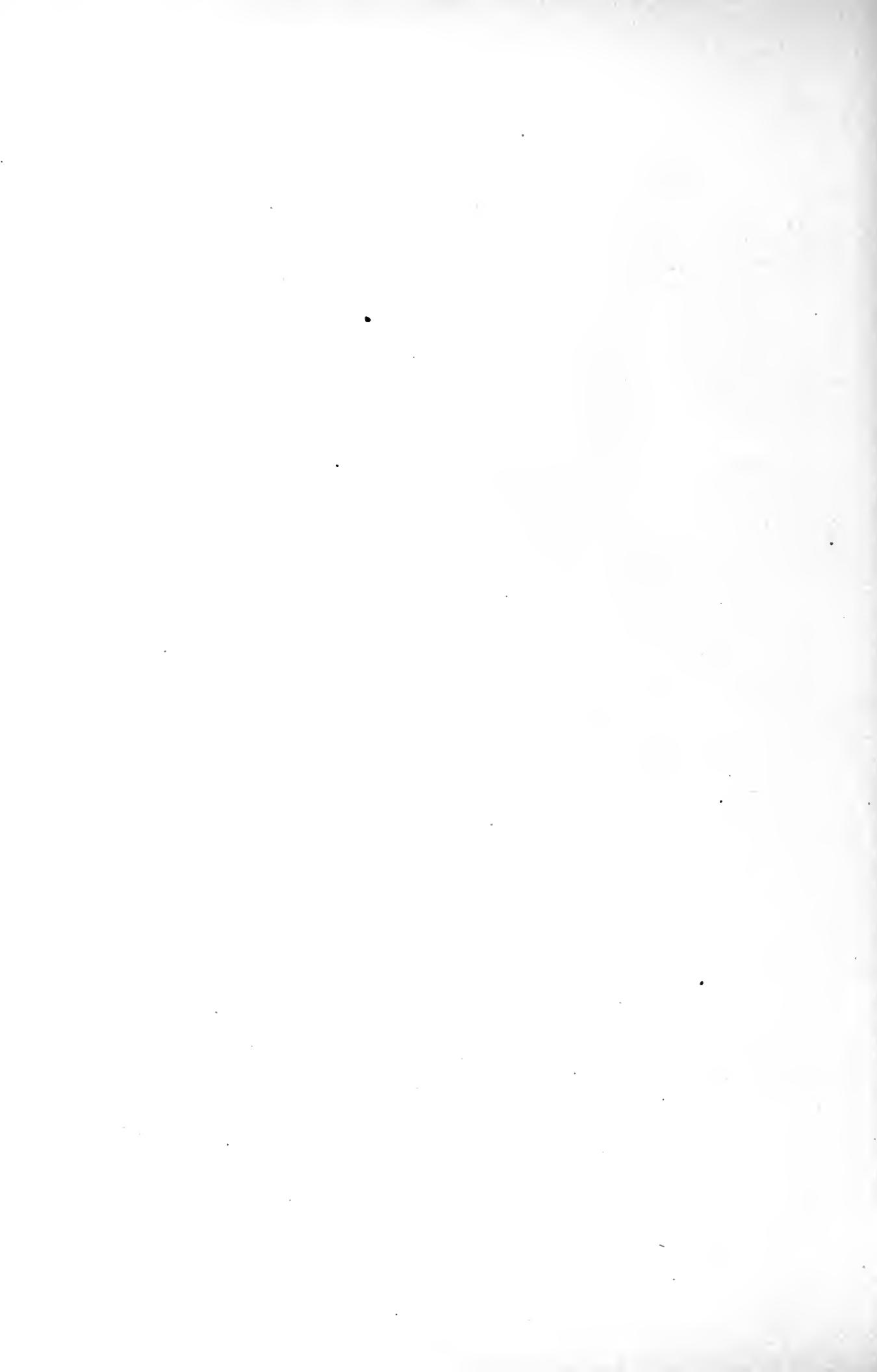
Figs. 24, 25.—Lower cap much broader than the upper; the granular matter within the nucleus is derived largely from the nucleolus and stains with gentian violet.

FIG. 26.—Late prophase; the achromatic figure evidently consists of two half-spindles.

FIG. 27.—Spindle very broad at the poles; a rather common form at this stage in the first three mitoses; no radiations or centrospheres.

FIG. 28.—The caps have kept the ends of the spindle rounded for an unusually long period; no centrospheres or radiations.

FIG. 29.—Telophase; radiations, but no centrospheres have reappeared.



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PLATE XXV

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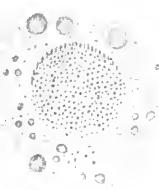
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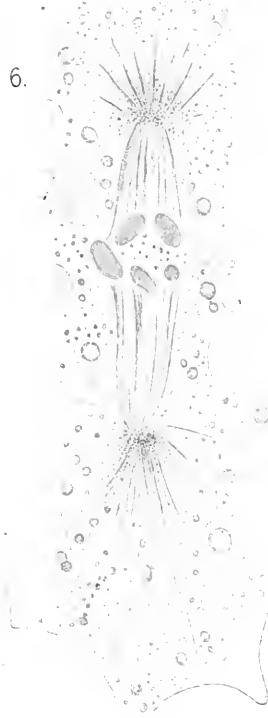
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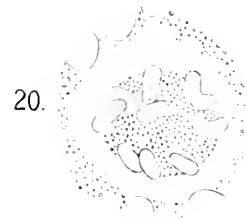
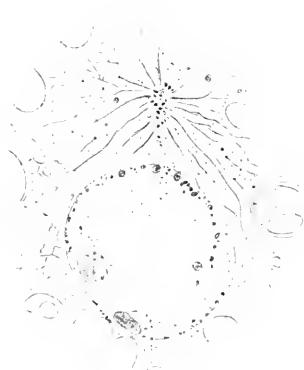
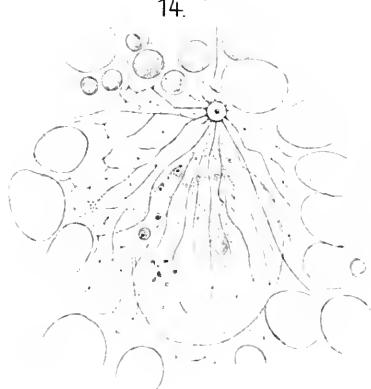
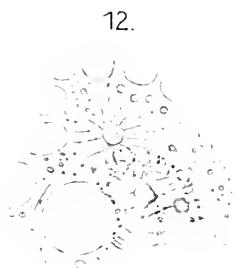
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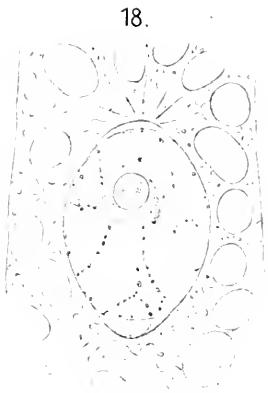


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PLATE XXVI



19.





DECENNIAL PUBLICATIONS, X

PLATE XXVII

21.



22.



23.



24.



25.



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27.



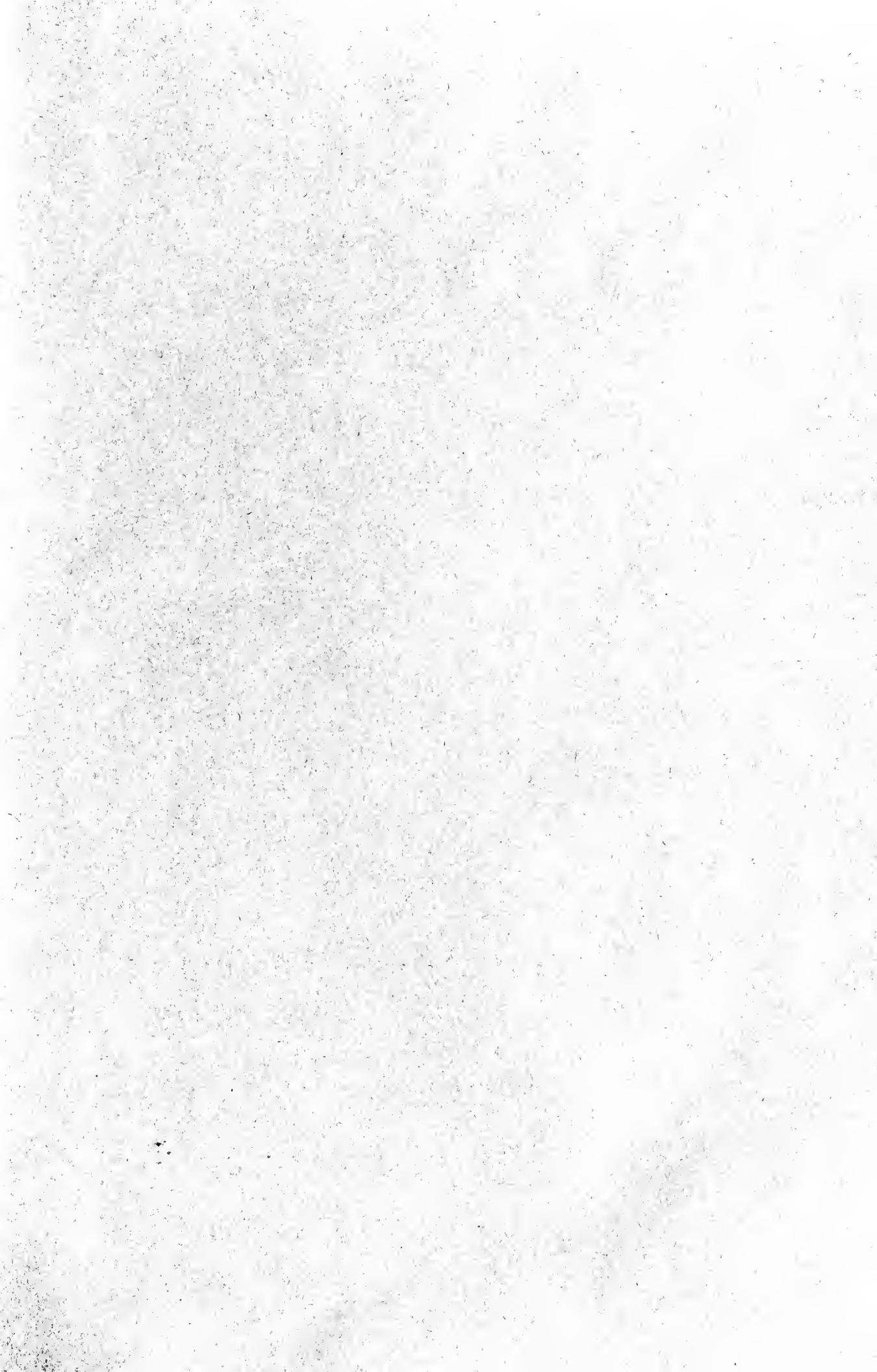
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